Novel Therapies in AML: Reason for Hope or Just Hype?

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OVERVIEW

We have entered the genomic sequencing era in the treatment of acute myeloid leukemia (AML); our patients increasingly and justifiably demand personalized treatment based on aberrations of their own leukemia. Except in rare cases we are not yet able to provide truly personalized therapy, so the question of “hope or hype?” posed by the American Society for Clinical Oncology (ASCO) for this educational topic is quite timely. The answer based solely on advances in genomic sequencing is “both”. There is an element of expectation among the public that we are “almost there” in solving the genetic cancer puzzle, an expectation indeed based on hype. However, there is no question that ultimate success lies in understanding the genetic underpinnings of disease. When decades of research in molecular biology and immunology are combined with transformative advances in cancer genetics, the answer is undeniably that our patients finally have reason for hope. Here, we review selected novel therapies for AML in areas such as immunotherapeutics, epigenetics, kinase inhibition/pathway inhibition, and the marrow microenvironment.

The graft-versus-leukemia (GVL) effect after allogeneic stem cell transplantation (alloHCT) demonstrates the potential of the immune system to recognize and eliminate (minimal) residual AML. AlloHCT remains the most powerful therapy available to reduce the risk of relapse, and the only cure for those refractory to current chemotherapies.1 However, toxicities associated with transplantation including graft-versus-host disease (GVHD) and limitations in donor availability remain major roadblocks to providing this option for more patients. Indeed, the Seattle group recently reported that only 60% of eligible patients actually undergo transplant.2 Advances in alloHCT for patients with AML are reviewed by Dr. Fred Appelbaum in this 2014 ASCO Education Book. AlloHCT successes such as the disease-free survival benefit seen in older patients with AML who are transplanted in the first complete remission (CR1),3 increasing use of alternative donors (haploidentical matches or umbilical cord blood),4 and natural killer (NK) cell inhibitory receptor mismatching between donor and recipient to increase GVL,5 further deepen enthusiasm for harnessing the potential of the immune system to treat AML. Understanding that GVL is instrumental in the curative potential of alloHCT begs the question of how to harness a potent immunologic response in the patient with AML without having to go through the entirety of a transplant, and with less risk. The potential is enormous; cancer immunotherapy was recently named “breakthrough of the year” by Science journal.6 Among these were recent studies of recombinant interleukin-2 (rIL-2), with or without histamine dihydrochloride, during maintenance. One study with rIL-2/histamine dihydrochloride showed a statistically significant disease-free survival benefit (p = 0.01) but no benefit for overall survival, and several other trials were negative.7-13 A meta-analysis of rIL-2 maintenance trials showed no benefit.14 Nonetheless, new opportunities to treat leukemia by reversing “leukemia-related immunosuppression” abound.15 Autologous T cells and NK cells may be able to eliminate disease by enhancing antitumor surveillance. Colleagues specializing in solid tumor oncology have led the way using monoclonal antibodies (mAbs) that target T cell immune checkpoint blockade to reverse immunologic tolerance of cancer cells. Foremost among these T-cell targets are cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed death-1 (PD-1); mAbs targeting these checkpoints are active in metastatic melanoma when given alone, in combination, and even after failure of the other agent.16-18 These agents are now entering clinical trials for AML. Although there are only a handful of AML trials with these agents currently listed at clinicaltrials.gov (such as NCT01096602, an ongoing trial with PD-1 blockade plus dendritic cell vaccine), the number of investigations in AML (and other hematologic malignancies) will likely increase rapidly in the coming 18 months. Similarly, novel mAbs designed to enhance NK cell anticancer immunity are attractive options. Antibodies targeting NK immunoglobulin-like receptor (KIR) showed ac...
tivity in early trials for patients with AML, myeloma, and lymphoma. A phase I trial with maintenance therapy in AML with IPH2101 (lilumab, an anti-KIR antibody) was recently completed and demonstrated the feasibility of KIR blockage and tolerability of the drug. A randomized phase II efficacy study as maintenance for older patients with AML in remission is now ongoing in Europe (ALFA/GOELAMS cooperative groups, NCT01687387). Preclinical studies suggest that blocking CD200 on leukemia cells can also reverse NK antitumor tolerance; CD200 is known to be upregulated on AML cells and suppresses NK function, providing a potential means of immunologic escape.

Breaking phagocyte tolerance of AML cells also may have a therapeutic role. Reversal of this so-called “do not eat me” signal has been successfully achieved in preclinical models. CD47 on leukemic stem cells (LSC) appears to be an important target in this pathway. CD47 on AML cells binds to the inhibitory signal-regulatory-protein-alpha (SIRPα) receptor on macrophages. The CD47-SIRPα interaction defends the myeloid blast by inducing tolerance in the macrophage, ultimately inhibiting phagocytosis. Weissman and others are developing mAbs that target this interaction. Likewise, fusion proteins have been engineered to combine macrophage-derived SIRPα with different human Ig Fc regions (SIRPαFc) thus creating a drug that binds CD47 with the specificity of a naturally occurring ligand but induces phagocytosis instead of tolerance (Table 1).

**Monoclonal Antibodies and Similarly Targeted Immunotherapeutics**

Antibodies as tumor-targeting therapies have been under investigation since the early 1980s, and there is an ever-growing population of U.S. Food and Drug Administration (FDA)-approved mAbs that directly target cancer-associated proteins (HER2/neu, EGFR, VEGF, CD20, CD52, and CD33) for the treatment of solid and hematologic malignancies. The most promising antibodies currently under investigation for AML are directed toward the myeloid precursor marker CD33 and the putative LSC-associated CD123.

CD33 is present on myeloblasts in 85% to 90% of cases of AML. This surface marker is generally restricted to early multilineage hematopoietic progenitors and absent from normal pluripotent hematopoietic stem cells. Unfortunately, the landscape of CD33 mAbs is littered with more failures than successes, including lintuzumab and gemtuzumab ozogamicin (GO). Lintuzumab is an unconjugated, fully-humanized anti-CD33 mAb with modest single-agent activity in AML; it did not improve patient outcomes in a randomized controlled trial when added to conventional chemotherapy. It was, however, noted to be rapidly internalized, suggesting a potential method of delivering cytotoxic therapy to CD33+ cells. To exploit this, Hammand and collaborators designed the first antibody-drug-conjugate (ADC) for AML, GO, which is an anti-CD33 mAb conjugated to the antitumor antibiotic calicheamicin. GO was approved by the FDA in 2000 through an accelerated approval process only to be withdrawn from the market in 2008. Several trials in North America (SWOG 106) or Europe (MRC 1113, among others) incorporated GO into induction with conventional chemotherapy but with increased induction mortality in the GO arm (SWOG 106) and uncertain clinical benefit (both trials). However, the drug’s regulatory future may not be dead yet, because subsequent subset analyses of the randomized trials and a recent meta-analysis of five trials combining GO with standard therapy found a meaningful benefit with GO in favorable risk patients (hazard ratio [HR] 0.50) and intermediate (HR 0.85) risk patients, but not in adverse risk patients (HR 1.04). To paraphrase Dr. Alan Burnett’s summary of the treatment effect from the MRC trial, “70% of patients get 10% benefit”. Regardless of whether GO comes before the FDA again, a great deal has been learned about mAb therapy in AML from these trials. Similar technology is being used with success in other diseases, such as inotuzumab in acute lymphoblastic leukemia (ALL). Past work informs the next generation of mAbs coming into the clinic now, among which is SGN-33A. Because the high incidence of liver toxicity with GO was attributed to the unstable linker, SGN-CD33A was engineered with a revised protease-cleavable linker. This ADC is an anti-CD33 mAb attached to a pyrrolobenzodiazepine dimer that is capable of causing DNA crosslinking and cell death in primary AML cells at a 3-fold increased potency compared with GO; SGN-CD33A is currently in clinical trials.

Although CD33 is present on the majority of AML blast cells, it is not present on LSCs. The exact immunophenotype of the LSC is still under debate; however, CD123 (IL-3R) is consistently overexpressed on LSCs as well as leukemic blasts and is a promising therapeutic target for AML. Several therapies targeting this protein already exist, including SL-401, a novel biologic directed to the IL-3 receptor that con-
sists of IL-3 conjugated to a truncated diphtheria toxin. SL-401 is being developed to treat the myeloid neoplasm blastic plasmacytoid dendritic cell neoplasm (BPDCN) in addition to AML. In data presented at ASH 2013, an impressive 4 out of 5 patients with this rare disease (BPDCN) achieved complete response (CR) with single agent SL-401, the remaining 1 out of 5 had a partial response (PR).39 A phase I/II trial examined the use of SL-401 as a single agent in patients with AML who had relapsed/refractory disease, or were unfit for standard treatment, demonstrated less encouraging responses; among 70 patients, there were two durable CRs and five PRs.40

Next-generation mAbs target not only the tumor but also effector cells to enhance their activity. One such compound is

### TABLE 1. Immunotherapeutics in Development

<table>
<thead>
<tr>
<th>Target(s)</th>
<th>Stage of development</th>
<th>Efficacy data</th>
<th>Ongoing trials</th>
<th>Company</th>
</tr>
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<tbody>
<tr>
<td><strong>Monoclonal Antibodies</strong></td>
<td></td>
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<tr>
<td>CSL362 CD123 (IL-3R)</td>
<td>Phase I</td>
<td>Preclinical elimination of LSC</td>
<td>NCT01632852</td>
<td>Janssen Biotech</td>
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<tr>
<td>4G8SDIEM FLT3</td>
<td>Upcoming Phase I/I</td>
<td>Compassionate use: 3/4 patients with elimination of MRD</td>
<td></td>
<td>University of Tuebingen</td>
</tr>
<tr>
<td>BMS-936564 CXCR4</td>
<td>Phase I</td>
<td>Mobilization of LSCs</td>
<td>NCT01120457, NCT01395657</td>
<td>Bristol-Myers Squibb</td>
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<td><strong>Antibody Drug Conjugate</strong></td>
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<tr>
<td>225-AcLintuzumab CD33</td>
<td>Phase I</td>
<td>4/6 patients with BM blast reduction; no CR</td>
<td>NCT00672165, NCT01756677</td>
<td>Actinium Pharmaceuticals</td>
</tr>
<tr>
<td>SGN-CD33A CD33</td>
<td>Phase I</td>
<td>Potent in vitro and in vivo activity, even with MDR and poor-risk cytogenetics</td>
<td>NCT0902329</td>
<td>Seattle Genetics</td>
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<tr>
<td>Gemtuzumab Ozogamicin CD33</td>
<td>Phase I/III</td>
<td>Increased relapse-free survival, primarily in good- to intermediate-risk patients</td>
<td>AAML0531 NCT01869803 NCT00091234</td>
<td>Pfizer</td>
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<td><strong>Targeting molecules</strong></td>
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<td>SIRPα-Fc fusion proteins CD47</td>
<td>Preclinical</td>
<td>Reduced leukemia burden in mouse models</td>
<td></td>
<td>Trillium Therapeutics</td>
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<tr>
<td>CD117-MTX Aptamer CD117</td>
<td>Preclinical</td>
<td>In vitro cell specific killing</td>
<td></td>
<td>The Methodist Hospital</td>
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<tr>
<td>SL-401(IL3-diphtheria toxin) CD123</td>
<td>Orphan Drug</td>
<td>Clinical CR/PRs</td>
<td></td>
<td>Stemline Therapeutics</td>
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<td><strong>DARTs</strong></td>
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<td>MG0006 CD123, CD3</td>
<td>Preclinical</td>
<td>Varying degrees of blast clearance in xenograft models</td>
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<td>MacroGenics</td>
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<td><strong>BITes</strong></td>
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<tr>
<td>AMG-330 CD33, CD3</td>
<td>Preclinical</td>
<td>CD33-dependent, dose proportional cell killing in vitro</td>
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<td>AMGEN Research</td>
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<td><strong>Triplebodies</strong></td>
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<td>SPM-2 CD123/CD33/CD16</td>
<td>Preclinical + case report</td>
<td>Ex vivo cytolytic activation of AML patient NK cells</td>
<td></td>
<td>Universität München</td>
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<td><strong>CAR T cells</strong></td>
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<tr>
<td>CD123</td>
<td>Preclinical</td>
<td>Elimination of AML cells +/− normal hematopoetic cells</td>
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<td>University of Pennsylvania, Beckman Research Institute</td>
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<td>CD33 Phase I</td>
<td>In vitro CD33-specific cytotoxicity</td>
<td></td>
<td>NCT01864902</td>
<td>St. Jude’s</td>
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<tr>
<td>LeY Phase I</td>
<td>4 patients: tolerability, some reduction in blasts, persistence of clone to 10 months</td>
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<td>University of Melbourne</td>
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<td><strong>Vaccines</strong></td>
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<tr>
<td>DC WT1</td>
<td>Phase I-II</td>
<td>Relapse prevention</td>
<td>NCT01483274, NCT00834002</td>
<td>University Hospital, Antwerp University of Louisville</td>
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<tr>
<td><strong>TLR-DCs</strong></td>
<td>Multiple</td>
<td>Superior T cell/NK cell activation</td>
<td></td>
<td>Tehran University</td>
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<tr>
<td><strong>DC/AML fusion</strong></td>
<td>Preclinical</td>
<td>Expansion of bone marrow infiltrating AML reactive T-cells</td>
<td>NCT01096602</td>
<td>Beth Israel Deaconess</td>
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Abbreviations: LSC, leukemic stem cell; MRD, minimal residual disease; BM, bone marrow; CR, complete response; PR, partial response; NK, natural killer.
AMG-330, a novel CD33-CD3 bispecific T-cell engager (BiTE antibody), a single-chain molecule composed of two variable chain specificity domains, CD33 and CD3. The CD19-CD3 BiTE blinatumomab has been very successful in early clinical trials for B-cell ALL.\(^{41}\) In vitro studies of AMG-330 with primary AML cells showed potent cytotoxicity regardless of the degree of CD33 expression.\(^{42}\) Of note, AMG-330 did not reduce surface expression of CD33, and its cytotoxic effects were potentiated by panobinostat and azacitidine.\(^{43}\) MGD006 represents another approach to combined antibody targeting and T-cell directed killing of AML cells. MGD006 is a dual affinity retargeting molecule (DART) with specificity for both CD123 and CD3. These complexes consist of two individual chains, each with a VH of one antibody in tandem with a VL of the other, which heterodimerize to form a single compound that acts as a magnet between DCs and CD3 cells. Preclinical studies showed marked T-cell activation, expansion of memory T cells, and cell killing that was specific to CD123 expression. Furthermore, the drug produced dose-dependent cytotoxicity in AML samples but had no effect on cord blood colonies, suggesting that normal stem cells may not be affected.\(^{44}\)

If a bi-specific molecule has increased activity over a mAb, is a trispecific molecule even better? The answer to that question is of course unknown, but one group has designed and evaluated such a molecule in a single-patient case study. Braciak et al. described a single chain tripeptide (SPM-2) that contains single-chain antibody fragments (scFv) to two AML cell surface markers (CD123, CD33) plus CD16 (found on NK cells and some macrophages). In a single-patient case study, the authors looked at NK cell expression at presentation and at remission compared with a monozygotic twin and found that ex vivo coadministration of SPM-2 with autologous NK and AML cells resulted in increased cell lysis (80% with SPM2 plus NK, 15% with NK alone). The number of LSCs was also markedly reduced with SPM2 plus NK cells compared with NK cells alone. Although only a case report, this suggests that NK cells may be effective for cell-mediated cytotoxicity in AML, and that the trispecific molecule may target the LSCs and facilitate a cure in the setting of minimal residual disease (MRD).\(^{45}\)

**Vaccines**

Creating a vehicle for in vivo T-cell expansion and disease-specific immunity that could be maintained over time is an attractive and long-investigated possibility. Anticancer vaccine therapy has been tried in a variety of malignancies without overwhelming success. Nevertheless, efforts have increasingly focused on applying this technology to patients with minimal disease burden after initial therapy, rather than untreated disease or frank relapse. Several promising new vaccine approaches were presented at the 2013 annual meeting of the American Society of Hematologists (ASH). Lichtegnegger et al. from the Helmholtz Institute in Munich have refined the process of maturing dendritic cells (DCs) to maximally stimulate naive T cells; their ex vivo studies explored various antibodies for T-cell checkpoint blockade in the vaccination process.\(^{46}\) Rosenblatt’s group in Boston showed that coculturing AML blasts and DCs to create fusion dendritic cells is feasible and active in vivo.\(^{47}\) This process allowed antigen presenting cells to display a wide array of tumor antigens, and was well tolerated without any measurable autoimmunity in patients with AML in complete remission who were deemed unsuitable for alloHCT. Data presented at ASH 2012 showed that 9 of 13 evaluable patients (10 with nonfavorable prognosis) remained in remission at 23 months of follow up, with good tolerability.\(^{48}\) Additional clinical studies employ specific depletion of T regulatory cells before vaccination in patients with AML to assist in optimal vaccine response (NCT01513109, NCT01842139). Timing of vaccination, as well as overall burden of disease, will likely play crucial roles in the success and direction of future vaccination therapies.

**Chimeric Antigen Receptor Immune Cells**

Chimeric antigen receptors (CARs) were first developed over 25 years ago but have not been clinically relevant until recently. The principal of development was to harness T-cell cytotoxicity and resiliency while removing the constraints of the T-cell receptor (TCR), namely the restriction to major histocompatibility complex interactions and the potential for coinhibitory signaling. This task is accomplished by replacing the TCR with a similarly specific moiety such as an antibody fragment. This opens up the possibility of targeting a wider array of cell surface markers. At a practical level, T cells are modified using gene splicing techniques to display CARs, which have an external domain to target a specific antigen that attaches to a transmembrane domain via a hinge linker and an intracellular domain with a CD3 signaling region. Second- and third-generation CARs will have additional intracellular signaling domains that can increase the activity of the CAR T cell by circumventing the T cell’s need for costimulatory molecules, which are often absent in malignancy.\(^{49}\) Powerful effects have been seen with second-generation CAR T cells targeting CD19, with CRs in refractory chronic lymphocytic leukemia (CLL) and ALL.\(^{50-52}\) Unfortunately, in some cases treatment was accompanied by a life threatening cytokine storm, which has since been ameliorated with IL-6 signaling blockade. Whether the successes seen in B-cell leukemias will be replicated in myeloid diseases remains to be seen. Mardiros et al. designed two novel second-generation CAR T cells specific to CD123, complexed with CD3 and CD28 signaling domains. In vitro studies demonstrated the ability to distinguish between CD123+ and CD123− cells, with the ability to kill primary AML cells with high-level expression of CD123. Researchers were also able to isolate monocytes from primary AML samples to create autologous CD123 CAR T cells.\(^{53}\) In a similar fashion to CAR T cells, Tettamanti et al. showed that cytokine-induced killer cells that express CD123 CAR induce cell death in CD123+ transduced cell lines with relative sparing of endothelial cells, monocytes, and hematopoietic stem cells (all of which express lower levels of CD123).\(^{54}\) Heiber et al. showed that anti-CD33 CAR cytotoxic lymphocytes spe-
Epidemiology

The epidemiology of AML is characterized by a relatively low incidence compared to other hematopoietic malignancies. In the United States, AML affects approximately 15,840 new cases annually, with an estimated 6,220 deaths due to the disease. The majority of cases occur in adults, with younger people accounting for a small percentage. The risk of AML increases with age, with a peak incidence in the 65-74 age group. Risk factors for AML include prior chemotherapy, radiation therapy, and exposure to certain chemicals or agents. Genetic and familial factors also contribute to the development of AML.

Risk Factors

Risk factors for AML include prior chemotherapy or radiation therapy, exposure to certain chemicals or agents, genetic predispositions, and familial factors. The incidence of AML is higher in men than in women, and the risk increases with age. Other risk factors include receipt of stem cell transplants, certain occupational exposures, and genetic predispositions such as Down syndrome.

Pathogenesis

The pathogenesis of AML involves the clonal evolution of a hematopoietic stem cell, leading to the accumulation of genetic mutations that confer a growth advantage to the leukemic cells. The hallmark of AML is the presence of leukemic cells that are dysregulated, exhibiting rapid proliferation and impaired differentiation. These cells accumulate in the bone marrow, leading to anemia, thrombocytopenia, and leukocytosis.

Genetic Mutations

Genetic mutations play a crucial role in the pathogenesis of AML. Mutations in driver genes, such as FLT3-ITD, NPM1, and CEBPA, are present in up to 30% of AML cases. These mutations can lead to alterations in signaling pathways, promoting leukemic cell proliferation and inhibiting differentiation. Other genetic alterations, such as epigenetic changes, chromosomal translocations, and microRNA dysregulation, also contribute to the pathogenesis of AML.

Clinical Presentation

The clinical presentation of AML is characterized by symptoms related to anemia, thrombocytopenia, and leukocytosis. Common symptoms include fatigue, weakness, easy bruising, and mucosal bleeding. Patients may also present with fever, night sweats, or weight loss. Physical examination may reveal hepatosplenomegaly and pallor. Laboratory findings typically show a low hemoglobin level, low platelet count, and a marked increase in white blood cells, often with a blastic morphology.

Diagnosis

The diagnosis of AML is made through a combination of clinical presentation, laboratory tests, and bone marrow examination.Peripheral blood examination may show anemia and leukocytosis. Bone marrow aspirate and biopsy reveal a hypercellular marrow with dysplastic changes and an increase in blast cells. Cytogenetic analysis and molecular testing are essential for identifying specific genetic abnormalities.

Treatment

The treatment of AML is multidisciplinary and depends on the specific subtype, patient characteristics, and disease stage. The mainstays of treatment include chemotherapy, targeted therapy, and hematopoietic stem cell transplantation. Induction chemotherapy, typically consisting of cytarabine and anthracyclines, is used to induce remission. Maintenance therapy may include additional chemotherapy or targeted agents. Patients with specific genetic mutations, such as FLT3-ITD and NPM1, may benefit from specific inhibitors.

Supportive Care

Supportive care is an integral part of the management of AML. It includes blood transfusions, antibiotics, anticoagulation, and platelet transfusions to manage anemia, infection, and bleeding. Gastrointestinal prophylaxis is also important to prevent mucositis and other side effects of chemotherapy.

Prognosis

The prognosis of AML is determined by several factors, including age, performance status, and the presence of specific genetic mutations. Patients younger than 60 years with favorable cytogenetics and normal karyotype have a better prognosis compared to older patients or those with unfavorable cytogenetics. The overall survival rate for untreated AML is approximately 10%, with 5-year survival rates ranging from 25% to 30% for patients receiving standard therapy.

Future Directions

Advances in the understanding of AML pathogenesis have led to the identification of novel therapeutic targets. Ongoing research is focused on developing more effective and less toxic therapies. Emerging strategies include the use of precision medicine, immune checkpoint inhibitors, and epigenetic modulators. Novel therapeutic approaches are also being explored, such as CAR T-cell therapy and localized radiotherapy, which may offer improved outcomes for patients with AML.

References

1. Leukemia and Lymphoma Society. AML. Available at: https://www.lls.org/cancer-types/acute-myeloid-lymphoma
States with encouraging preliminary results (Table 2). KIT (CD117) has been targeted not only by tyrosine kinase inhibitors (e.g., dasatinib) but also by novel means. Aptamers are single-stranded DNA or RNA oligonucleotides with a tertiary structure that has binding specificity to its target, similar to an antibody but more easily synthesized and less immunogenic. Zhao et al. designed an aptamer-drug conjugate targeting KIT (CD117-methotrexate) that showed specific killing of CD117+ AML cells in vitro. Such novel approaches are not quite ready for patient trials, but given their ease of manufacture and predicted tolerability, if found to be efficacious they may open the door to more rapid preclinical development for many targets beyond KIT.

The human Aurora kinase family (A,B,C) is a highly conserved group of Src-threonine kinases that play a role in mitosis. Aurora A kinase has been linked to carcinogenesis in humans and Aurora B mediates chromosomal packaging during mitosis and has been targeted successfully in AML; inhibition of Aurora B with AZD1152 had a 25% response rate with 9/64 CRs in a poor-risk AML population.

Interest in Aurora kinases as targets continues, and recent data on AMG 900 indicated nanomolar efficacy against 10 AML cell lines, as well as in vivo responses in a murine model. Similarly, polo-like kinases (Plks) play an important role in cell cycle progression and have been targeted in AML. A randomized phase II study of volasertib (BI 6727), an intravenous Plk inhibitor, was conducted in older patients with AML who were not candidates for intensive therapy. The patients received low-dose cytarabine (LDAC) with or without volasertib. Response was seen in 31% of patients (13 of 42) treated with the combination versus 13% of patients (6 of 45) treated with LDAC alone (p = 0.0523), and overall survival was 8.0 months with the combination versus 5.2 months with LDAC alone (p = 0.0996). These results led to Breakthrough Therapy designation by the FDA and initiation of POLO-AML-2, a phase III randomized trial of the same regimens in patients with AML who are aged 65 or older and ineligible for intensive therapy.

### TABLE 2. Newer Targeted Therapies and Trial Combinations

<table>
<thead>
<tr>
<th>Molecular Targets</th>
<th>Individual Drugs</th>
<th>Combinations</th>
<th>Efficacy</th>
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<tbody>
<tr>
<td>Epigenetic</td>
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<tr>
<td>DNMT</td>
<td>decitabine, SGI-110, vidaza</td>
<td>KPT330+decitabine</td>
<td>Enhanced transcription + decreased nuclear transport (XPO1) improves median survival in mice&lt;sup&gt;33&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDAC</td>
<td>vorinostat, panobinostat</td>
<td>plerixafor + decitabine</td>
<td>ORR 43%, Median OS for responders 18 mos, nonresponders 5 mos (P&lt;0.001)&lt;sup&gt;33&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD1</td>
<td>GSIG287652</td>
<td>dasatinib + cytarabine + daunorubicin</td>
<td>Phase II: de novo CBF-AML, CR 92% (96% age 60 or less, 80% age &gt;60)&lt;sup&gt;34&lt;/sup&gt;</td>
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<td>DOT1L</td>
<td>EP2-5676</td>
<td>MK-2206 + rapamycin + BEZ235</td>
<td>Delayed tumor progression and prolonged OS in xenograft mouse model&lt;sup&gt;36&lt;/sup&gt;</td>
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<tr>
<td>IDH1/2</td>
<td>AG1-14100/AG221</td>
<td>panobinostat + JQ1</td>
<td>Combination shows superior survival mice, as compared to treatment with JQ1 or PS alone (p &lt; 0.001)&lt;sup&gt;36&lt;/sup&gt;</td>
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<tr>
<td>BRD4</td>
<td>CTX015</td>
<td>vorinostat + idarubicin + cytarabine</td>
<td>De novo(N = 26), ORR = 88% Re/Ref (N = 13), ORR = 30%&lt;sup&gt;37&lt;/sup&gt;</td>
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<td>Protein Kinases</td>
<td></td>
<td>BL-8040 + cytarabine</td>
<td>96% reduction in cell viability and inducing cell death by 70-90% of AML cells in vitro&lt;sup&gt;38&lt;/sup&gt;</td>
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<td>c-KIT</td>
<td>PLX3397, dasatinib</td>
<td>BL-8040 + cytarabine or AC220</td>
<td>BMS-936564 + MEC (mitoxantrone, etoposide, cytarabine)</td>
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<td>FLT3</td>
<td>E6201, PLX3397, tandutinib, sorafenib, sunitinib, midostaurin, lestaurtinib, crenolinib, KW-2449, quizartrin (AC220)</td>
<td>MK-2206 + rapamycin + BEZ235</td>
<td>Delayed tumor progression and prolonged OS in xenograft mouse model&lt;sup&gt;36&lt;/sup&gt;</td>
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<td>Aurora Kinase</td>
<td>AMG 900, MLN8054</td>
<td>Combination shows superior survival mice, as compared to treatment with JQ1 or PS alone (p &lt; 0.001)&lt;sup&gt;36&lt;/sup&gt;</td>
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<tr>
<td>MEK1</td>
<td>E6201</td>
<td>Panobinostat + JQ1</td>
<td>Combination shows superior survival mice, as compared to treatment with JQ1 or PS alone (p &lt; 0.001)&lt;sup&gt;36&lt;/sup&gt;</td>
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<td>PISK/Akt/mTOR</td>
<td>BEZ235, MK-2206, rapamycin</td>
<td>MK-2206 + rapamycin + BEZ235</td>
<td>Delayed tumor progression and prolonged OS in xenograft mouse model&lt;sup&gt;36&lt;/sup&gt;</td>
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<tr>
<td>HCK</td>
<td>PK-20449</td>
<td>MK-2206 + rapamycin + BEZ235</td>
<td>Delayed tumor progression and prolonged OS in xenograft mouse model&lt;sup&gt;36&lt;/sup&gt;</td>
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<td>Mitochondrial</td>
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<tr>
<td>Bcl-2</td>
<td>JQ1, 1-BET151, ABT-199, ABT-737</td>
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<td>Caseinolytic protease</td>
<td>A2-32-01</td>
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<tr>
<td>MDM2</td>
<td>RG7112, RO5603781</td>
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<td>BM Environment</td>
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<tr>
<td>CXCR4</td>
<td>BL-8040, plerixafor, BMS-936564</td>
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<tr>
<td>CXCL12</td>
<td>NOX-A12</td>
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<td>Other</td>
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<tr>
<td>XPO1</td>
<td>KPT-330</td>
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Abbreviations: ORR, overall response rate; CR, complete response.
ALTERNATIVE TARGETS

Despite the molecular heterogeneity of AML, there is hope for a common target that would have broad applicability. One such target is the nuclear exportor CRM1/XPO1. XPO1 is a subclass of karyopherin that facilitates the nuclear-cytoplasmic transport of over 200 proteins and several mRNAs. It is the sole nuclear exporter of p53, p73, FOXO, IkB/NF-kB, Rb, p21, and nucleophosmin. In the class of selective inhibitors of nuclear export (SINE), selinexor (KPT-330), a novel, irreversible inhibitor or XPO1, has emerged as an active therapy in AML. After 35 days of taking this oral medication, primagraft mice had normalization of their bone marrow and a repopulation assay showed a decrease in LSCs of 6-fold in FLT3-ITD AML and 100-fold in complex karyotype AML. In an initial report of an ongoing phase I study including 14 patients, 2 (14%) achieved CR with single agent selinexor, another two showed CRi, and four had stable disease after more than 30 days. Side effects were most commonly anorexia (which could be quite severe), nausea, and fatigue.72-73 Several studies of selinexor in combination with epigenetic or conventional therapies are planned. Along the same lines, MDM2 (murine double minute 2 protein) is an attractive “broad” target in AML because it inhibits transactivation of p53, facilitates its export out of the nucleus, and promotes its degradation through ubiquitination.74-76 Surprisingly, CR was achieved in 16% (5/31) of relapsed/refractory patients with AML in a single-agent phase I clinical trial of the MDM2 inhibitor RG7112.77 Follow-up phase lb data were presented in 2013, this time in combination with cytarabine (Arm A: 20 mg/m² daily for 10 days vs. Arm B: 1 g/m² for 6 days). Arm A had a 21% CR rate and Arm B had a 17% CR rate.78 A second-generation MDM2 inhibitor, RO5503781, is currently being tested as a single agent or in combination with cytarabine (NCT01773408).

Another target is Bcl-2. As an important regulator of apoptosis, Bcl-2 and related proteins have been tested in a multitude of cancers, including AML. Recently, a new Bcl-2 inhibitor, ABT-199, was tested in vitro against a range of AML samples. AML cells with diploid cytogenetics or with mutation of FLT3, NRAS, or NPM1 were treated with ABT-199 and 78% (18/23) were found to be sensitive to apoptosis; however, those with complex cytogenetics, t(8;21), and JAK2 mutation were resistant. Further investigation using NSG mice showed that treatment improved leukemia burden, decreased spleen size, and prolonged overall survival (p = 0.0004).79 Another group investigated ABT-199 using AML cell lines and patient samples and demonstrated efficacy with median IC50 of 20 nmol/L; they similarly found that efficacy was independent of NPM1 and FLT3 mutations and, in this case, also cytogenetics.80 A phase II clinical trial in AML is currently underway.

THE BONE MARROW MICROENVIRONMENT

The bone marrow niche may nurture LSCs and potentially shield these relapse-promoting cells from destruction. Most of the current focus in targeting the marrow microenvironment has been on the chemokine receptor CXCR4 and its interaction with SDF-1 (CXCL12), which is a homing signal to the bone marrow. It has been theorized that disrupting this interaction will displace LSCs from their protective environment, exposing them to therapeutic agents. Multiple CXCR4 antagonists have been developed; among them plerixafor is approved by the FDA to mobilize stem cells for autologous transplantation and is now being examined in conjunction with chemotherapy to capitalize on its potential to mobilize LSCs.81-83 More recently, BL-8040, a higher affinity CXCR4 antagonist, was shown to directly inhibit AML cell growth by 28% to 47% and increase cell death by 75% to 100%. When added to another therapy, either quizartinib or cytarabine, a 96% reduction in cell viability was observed.84 An alternative approach has been to focus on inhibition of the ligand, SDF-1. NOX-A12 is a novel SDF-1 Spiegelmer, a trademark molecule consisting of a structured L-RNA oligonucleotide that is PEGylated at the 5’ end to improve the pharmacokinetics. SDF-1 has been shown to impair chemotaxis of CLL cells and to enhance chemosensitivity in myeloma.85,86 In an AML murine xenograft model, the combination of CXCR4 inhibition (plerixafor) and SDF-1 (NOX-A12) resulted in an increased percentage of circulating blasts that was basically the sum of their individual effects.87 The clinical utility of these observations remains unclear.

MOVING FROM THE BENCH TO THE BEDSIDE

No review of novel therapies is complete without at least a brief look at how processes to develop drugs might be improved and accelerated. Computational modeling is a powerful tool to accelerate the development of therapies in cancer by helping to expedite and refine the drug discovery process. The creation of simulated environments to predict structure-based function or target-drug interactions is a new but expeditious means to derive new compounds or improve on already known drugs, or perhaps to deal with the ever-increasing number of novel targets (gene mutations). As an example, Chandran et al. used molecular docking studies to identify 3,088 compounds with desirable IC50 values as potential lead compounds.88 Another example of the potential power of these methods was demonstrated by Saito et al. in the development of RK-20449, a novel kinase inhibitor that is active in AML. They used in silico docking studies to identify 3,088 compounds that were structurally similar to known inhibitors of their target, hematopoietic cell kinase (HCK), out of almost 9 million commercially available compounds. HCK is a member of the Src family of kinases that is differentially expressed in AML LSCs. Enzymatic assays revealed three compounds with desirable IC50 values as potential lead compounds. They identified the most active compound based on enzymatic analysis and IC50 and returned to computational models to assess relative inhibition compared with known
inhibitors, as well as protein/ligand interactions. Their models predicted improved affinity compared to the known comparable small-molecule HCK inhibitor PP2, which held up in vitro. Unfortunately the new compound had poor solubility, so the data derived from the docking studies were used to make modifications to the starting compound to improve solubility while maintaining the critical protein/ligand interactions. Seven new compounds were synthesized and tested again, yielding RK-20449, a potent inhibitor of HCK, as a candidate for clinical studies in AML. In a particularly immunodeficient murine model (NSG mice) engrafted with resistant human AML, in vivo administration of RK-20449 virtually eliminated LSCs.

Additional methods such as bioinformatics and systems biology look to define mathematical relationships between structure and function. For any new drug needed, these enormous databases could hold the answers if the right question can be formulated. Other strategies focus on the personalization of therapy by applying gene sequencing to patients routinely to provide a unique and targeted approach to therapy.

CONCLUSION

The best available combination of therapy for AML today remains the same as in the 1970s: cytarabine and daunorubicin (“7+3”). However, advances in immunology and molecular biology, as well as bioinformatics and computational biology, are partnering with great leaps in genetic understanding to finally bring more effective therapies to the clinic. This review focused only on novel therapies for AML, yet there are too many options for us to discuss them all and we were not able to include many promising new drugs. For example, we cannot discuss all of the FLT3 inhibitors or all of the trials with nucleoside analogs. All of these novel approaches show great promise. Although it is perhaps still somewhat overly optimistic, it is no longer naive to believe that soon 7+3 will just equal 10. Hope springs eternal.


52. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-


91. The Cancer Genome Atlas Research Network. Genomic and epig-


